Proceedings

Visible-Near Infrared Platelets Count: Towards Thrombocytosis Point-of-Care Diagnosis†

Barroso TG, Ribeiro L [1,2], Gregório H [1], Santos F [3] and Martins RC [2,*]

[1] CHV - Veterinary Hospital Center, R. Manuel Pinto de Azevedo 118, Porto-Portugal
[2] Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisboa - Portugal
[3] INESC TEC - Institute for Systems and Computer Engineering, Technology and Science - Campus da FEUP, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
† Presented at the 1st International Electronic Conference on Chemical Sensors and Analytical Chemistry, 01—15 July 2021; Available online: https://csac2021.sciforum.net/.
* Correspondence: rmcm@inesctec.pt

Abstract: Thrombocytosis is a disorder with excessive number of platelets in the blood, being total platelet counts (TPC) crucial for diagnosis. This condition predisposes to blood vessels clotting and diseases such as stroke or heart attack. TPC is generally performed at the laboratory by flow cytometry with laser scattering or impedance detection. Due to limited capacity of automated hematology in performing TPC quantification, manual microscopy count is a very common quality assurance measure undertaken by clinical pathologists. Monitoring coagulation risk is key in many health conditions, and point-of-care platforms would simplify this procedure by taking platelet counts to the bedside. Spectroscopy has high-potential for reagent-less point-of-care miniaturized technologies. However, platelets are difficult to detect in blood by standard spectroscopy analysis, due to their small size, low number when compared to red blood cells, and low spectral contrast to hemoglobin. In this exploratory research, we show that it is possible to perform TPC by advanced spectroscopy analysis, using a new processing methodology based on self-learning artificial intelligence. Results show that TPC can be measured by visible-near infrared spectroscopy above the standard error limit of 61.19×10^9 cells/L (R2=0.7016), tested within the data range of 53×10^9 to 860×10^9 cells/L of dog blood. These results open the possibility for using spectroscopy as a diagnostic technology for the detection of high levels of platelets directly in whole blood, towards the rapid diagnosis of thrombocytosis and stroke prevention.

Keywords: Point-of-care; Spectroscopy; Platelets; Artificial Intelligence

Citation: . *Journal Not Specified* **2021**, *1*, 0.

Published: 01 July 2021

1. Introduction

Platelets (PLT) are the smallest cells in the blood, being responsible for coagulation and blood vessel repair. PLT counts reference interval in dogs is 300 to 500 $\times10^9$ cell/L. High PLT counts is a condition known as thrombocytosis, being attributed to abnormal bone marrow production or an ongoing condition such as anemia or inflammation [1]. Thrombocytosis can result in blood clots, leading to life-threatening or impairing conditions such as heart attack or stroke [2]. Automated PLT counts are mostly performed by flow cytometry, electric impedance (Coulter principle) or laser-scattering technologies [3]. However, these methods are prone to erroneous PLT counts, because of changes in cell size and morphology, due to blood clotting, activation, aggregation, or even post-sampling artifacts. This limits scattering angle and impedance detection, leading to misidentification as larger cells, such as erythrocytes or leucocytes. Laser scattering is significantly more accurate than electric impedance, but the latter is cheaper and has a higher implementation in Veterinary Medicine. Veterinary doctors make use of blood smear PLT manual counts for ensuring results quality in abnormal (low or high) values [4].

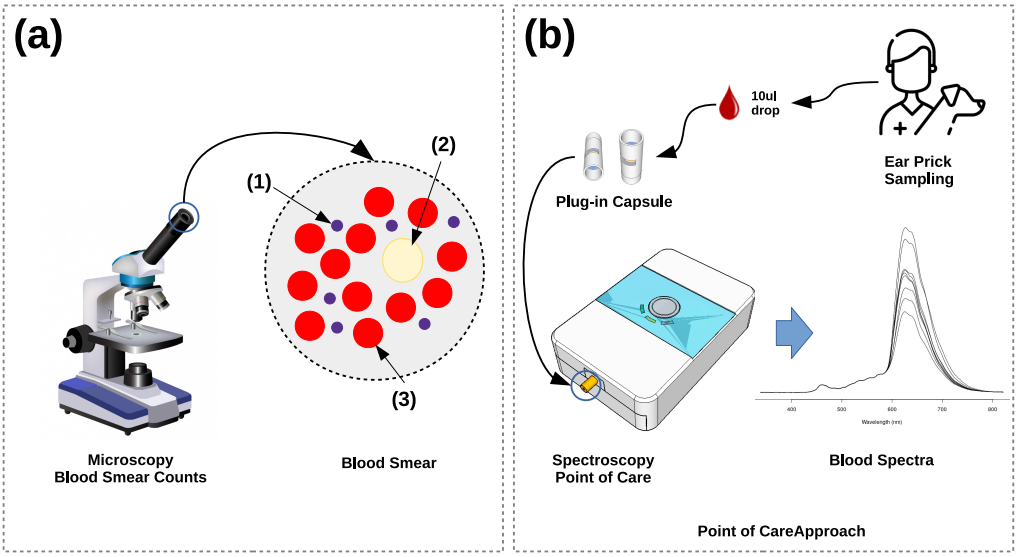

Figure 1. Platelets cell counts: (a) manual smear count at the microscope by trained hematologist demonstrating the proportionality between (1) platelets, (2) white blood cells and (3) red blood cells; and (b) Point-of-care approach - single blood drop spectroscopy counts using artificial intelligence.

Visible shortwave near-infrared (Vis-NIR) spectroscopy has a high potential for the development of point-of-care (POC) without the need for reagents or complex sample preparation. The developed Vis-SWNIR POC system (Figure 1b) records the blood spectra of a single drop of blood (<10 μL) to provide a significant number of clinical analysis parameters with real-time results [5].

Visible short-wave near-infrared (Vis-SWNIR) spectroscopy is an information-rich technology that carries both physical and chemical information, where the information about blood cells and constituents is distributed across the different wavelengths. Dominant spectral information in blood comes from highly absorbent constituents in the Vis-SWNIR region, such as hemoglobin present in red blood cells (RBC) and bilirubin in blood serum.

Platelets are present in significantly lower values than red blood cells (RBC) (Figure 1a). PLT reference interval in dogs is 300 to 500 $\times 10^9$ cells/L and RBC is 5500 to 8500 $\times 10^9$ cells/L, being at approximately 1:18 ratio to RBC, which difficults the detection:

i. Smaller size of PLT with the significantly lower area and volume for light absorbance, resulting in low sensitivity in the spectral signal;

ii. High interference between PLT and RBC, hemoglobin and bilirubin, which leads to the existence of significantly different characteristic interferences;

iii. High variance of PLT morphology - which can vary from small platelets to activated platelets with branches, and clotted cells.

PLT counts are difficult to obtain, even by microscopy methods, exhibiting high variability. Herein, we explore the capacity of Vis-SWNIR and self-learning artificial intelligence (SL-AI) for PLT quantification [5]. This new approach isolates spectral interference by searching consistent covariance between PLT and spectral features, which belong to a covariance mode (CovM). CovM is a set of samples that can hold a direct relationship between spectral features and PLT counts, by sharing a common latent structure [5]. Ideally, PLT counts are related to spectral interference features by a single latent variable (LV) or eigenvector. Such allows unscrambling the interference of PLT concerning the other blood constituents. This research provides a feasibility

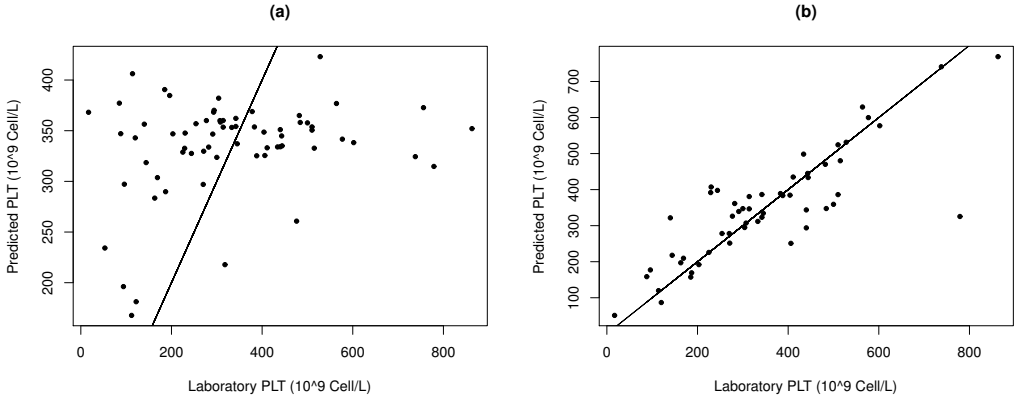

Figure 2. Total platelet counts spectral quantification: (a) PLS and (b) SL-AI.

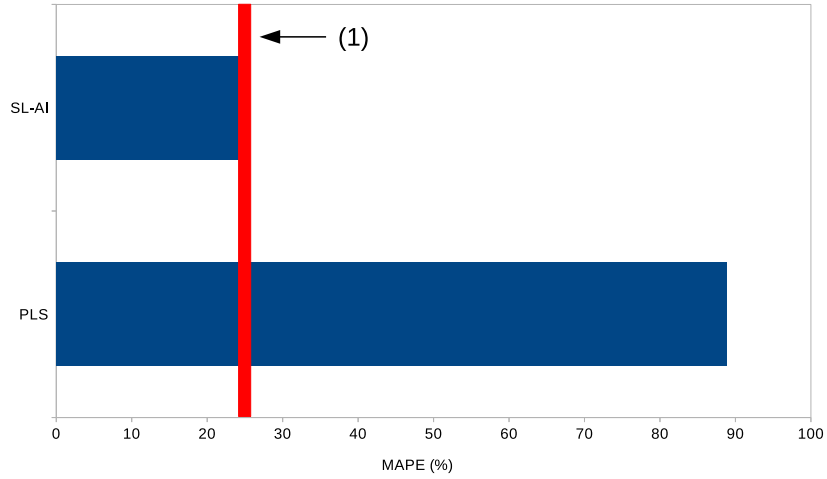

Figure 3. Percentage Total Error for PLS and SL-AI predictions: (1) ASVCP acceptable error limit (25%)

benchmark between the widely used chemometrics method partial least squares (PLS) and the SL-AI method.

2. Materials and Methods

2.1. Hemogram analysis

Dog blood samples from routine clinical practice were collected by qualified personnel by standard venipuncture, at the Centro Hopitalar Veterinário do Porto. PLT was determined by Beckman-Coulter capillary impedance using Mindray B-2800 vet auto-hematology analyzer.

2.2. Spectroscopy

Blood spectra were recorded using a POC prototype using a 4500K power LED as a light source and USB-based miniaturized spectrometer (Ocean Insight STS-vis), with an optical configuration and plug-in capsule system according to [6]. LED temperature and spectrometer integration times were automatically managed to maintain result consistency. Three replicate measurements were made for each blood sample.

2.3. Chemometrics

Spectral records were subjected to scattering correction (Mie and Rayleigh) before modeling. A feasibility benchmark is performed between PLS and SL-AI methods. PLS maximizes the global covariance between spectral features and PLT, by determining the orthogonal eigenvectors of the covariance matrix. The relationship between PLT and signal features is derived by the latent variables (LV), at each deflation. The number of LV is determined by cross-validation at the minimum value of the predicted residuals sum of squares (PRESS) [7].

SL-AI searches for stable covariance in spectral datasets, finding covariance modes (CovM). CovM is a group of samples that contains the same interference information characteristics, holding proportionality between PLT and spectral features. Ideally, the relationship between PLT and spectral features is given by a single eigenvector or latent variable (LV). The CovM is validated by leave one-out cross-validation [5].

3. Results and Discussion

PLS model attains a correlation of 0.2613 with a very poor R^2 (0.068), and a corresponding high SE of 175.99 $\times 10^9$ cells/l. The PLS analysis shows that the correlation between spectral features and PLT counts is highly unstable and non-linear. Such is because PLT is present in much fewer quantities than other blood constituents (Figure 1), as well as, due to the small size and high interference with the other major blood constituents (e.g. RBC, hemoglobin, and bilirubin). Another indication of non-linearity is that the PLS algorithm attains the optimum prediction error with two LV, resulting in a non-significant model (Figure 2a). The PLS is unable to increase the number of LV because the information about PLT is scattered in significantly different interference modes that cannot be collapsed into a linear oblique projection model [5,7]. PLS cannot be used in a POC as it does not attain a MAPE similar to 25% - the total allowable error established by the American Society for Veterinary Clinical Pathology (ASVCP) for PLT counts [8].

SL-AI present a significant correlation of 0.8376, a SE of 61.19 $\times 10^9$ cell/l, and MAPE of 24.67%, with R^2 of 0.7016 (Table 1). SL-AI covariance modes (CovM) are obtained with 1 to 3 LV. Such means that, although statistically valid relationships are obtained for each CovM, some of these are integrating more than one type of interference. Under ideal conditions, all CovM should have only one LV, directly relating PLT counts and spectral interference.

Results also show that non-dominant spectral information and low-scale spectral variation is unscrambled by the CovM principle. The number of LV can be attributed to the high diversity of PLT morphology present in dog blood (non-activated, activated, and clotted PLT) and particular conditions of the tested blood, with correspondence in the major constituents.

Despite the limitations shown in this feasibility study, PLT quantification using Vis-SWNIR spectroscopy in conjunction with the new SL-AI algorithm can attain a total error estimate of 25%. Such result is following the ASVCP total allowable error for PLT in dog blood [8].

Vis-SWNIR POC technology based on SL-AI has shown high potential for PLT quantification and thrombocytosis diagnosis. The results presented for dog blood are within the acceptable error defined by the ASVCP of 25% [8]. The presented results also allow extending the potential application to both human and other animal species in further studies.

4. Conclusions

This feasibility study has shown that low intensity, non-dominant, and multi-scale interferent spectral information is possible to be accessed, by unscrambling information with the CovM principle included in the SL-AI method. The small variations in the spectral signal that contain information about PLT cannot be modeled by PLS. SL-AI

Table 1. This is a table caption. Tables should be placed in the main text near to the first time they are cited.

Method	SE	LV	R^2	MAPE(%)	$R_{Pearson}$
PLS	175.99	2	0.068	88.89	0.2613
SL-AI	61.19	1-3	0.7016	24.67	0.8376

can unscramble PLT interference information based on the CovM principle, allowing the quantification of PLT. Future studies, with more samples, may provide better insights on the full potential of the developed POC technology in both veterinary and human medicine.

Author Contributions: **Barroso TG**, **Ribeiro L**, and **Gregório H**: Investigation, methodology, validation, writing - review & editing; **Santos F.**: investigation, hardware and firmware; **Martins RC**: conceptualization, software and hardware, funding acquisition, writing - original draft, resources and formal analysis, project administration.

Funding: Martins RC acknowledges Fundação para a Ciência e Tecnologia (FCT) research contract grant (CEEIND/017801/2018).

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Woolcock, A., Keenan, A., Cheung, C., Christian, J. and Moore, G. (2017), Thrombocytosis in 715 Dogs (2011–2015). J Vet Intern Med, 31: 1691-1699. https://doi.org/10.1111/jvim.14831
2. Soucy-Giguère, M. C., Turgeon, P. Y., Sènèchal, M. (2019). What cardiologists should know about essential thrombocythemia and acute myocardial infarction: report of two cases and advanced heart failure therapies considerations. International medical case reports journal, 12, 253–259. https://doi.org/10.2147/IMCRJ.S217568
3. Baccini, V., Geneviéve, F., Jacqmin, H., Chatelain, B., Girard, S., Wuilleme, S., Vedrenne, A., Guiheneuf, E., Toussaint-Hacquard, M., Everaere, F., Soulard, M., Lesesve, J. F., Bardet, V. (2020). Platelet Counting: Ugly Traps and Good Advice. Proposals from the French-Speaking Cellular Hematology Group (GFHC). Journal of clinical medicine, 9(3), 808. https://doi.org/10.3390/jcm9030808
4. Arnold, J.E., Camus, M.S., Freeman, K.P., Giori, L., Hooijberg, E.H., Jeffery, U., Korchia, J., Meindel, M.J., Moore, A.R., Sisson, S.C., Vap, L.M. and Cook, J.R. (2019), ASVCP Guidelines: Principles of Quality Assurance and Standards for Veterinary Clinical Pathology (version 3.0). Vet Clin Pathol, 48: 542-618. https://doi.org/10.1111/vcp.12810
5. Martins, R.C. Big data self-learning artificial intelligence methodology for the accurate quantification and classification of spectral information under complex variability and multi-scale interference. **2018**, WO/2018/060967, 5 April 2018.
6. Martins, R.C., Sousa, N.J., Osorio R. Optical system for parameter characterization of an element of body fluid or tissue. **2017**, US10209178B2, 19 Fev 2019 - Granted.
7. Geladi, P. Kowalsky, B. Partial least squares regression: a tutorial. *Anal. Acta*, **1988**, *185*, 1–17.
8. Nabity, M.B., Harr, K.E., Camus, M.S., Flatland, B., Vap, L.M. ASVCP guidelines: Allowable total error hematology. *Vet. Clin. Pathol.*, **2018** *47*, 9–21.